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Serum retinol levels are positively correlated with hemoglobin concentrations, independent of iron homeostasis: a population-based study[☆]

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ABSTRACT

Micronutrient interactions give rise to complex issues that have an impact on preventive strategies when multiple micronutrient deficiencies coexist. The aim of this population-based study was to determine the prevalence of vitamins A and E and iron deficiencies among women 15 to 49 years of age in the northern Persian Gulf region. We hypothesized that serum retinol levels may show correlations with hemoglobin (Hb) concentrations, independent of iron status. A total of 1242 nonpregnant women of reproductive age were selected via a multistage stratified random cluster sampling technique. Serum ferritin and soluble transferrin receptor levels were measured using enzyme immunoassay techniques. Serum retinol (vitamin A) and α -tocopherol (vitamin E) were determined for 727 women by high-performance liquid chromatography. The prevalence of anemia (Hb <12 g/dL), iron deficiency (serum ferritin <15 μ g/L), and iron deficiency anemia was 8.7%, 25.4%, and 4.6%, respectively. Vitamin A (<0.7 μ mol/L) and vitamin E (<11.6 μ mol/L) deficiencies were found in 1.2% and 5.9% of the studied population, respectively. Multiple regression analysis revealed that serum retinol levels exhibit a significant association with Hb concentrations after controlling for serum ferritin levels, anemia associated with chronic disease, and risk factors for anemia. Therefore, most nonpregnant women of reproductive age in the northern Persian Gulf were found to have adequate serum vitamin A and E levels. However, the status of anemia and iron deficiency anemia could be considered a mild public health problem in this region. On the basis of multivariate analyses, we conclude that low serum retinol levels may contribute to anemia, independent of iron homeostasis.

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Abbreviations: BMI, body mass index; Hb, hemoglobin; HPLC, high-performance liquid chromatography; IDA, iron deficiency anemia; sTfR, serum transferrin receptor; VAD, vitamin A deficiency; WHO, World Health Organization.

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1. Introduction

Micronutrient deficiencies are a major global health problem, resulting in significant social and economic costs, particularly in the developing world [1]. Micronutrient deficiencies rank among the 20 major risk factors for diseases in the poorest regions of the world, leading to morbidity and impaired quality of life [2,3]. Iron is an example of an essential micronutrient, necessary for erythropoiesis, oxidative metabolism, and cellular immunity [4]. Globally, anemia affects 24.8% of the population, and half of the cases of anemia are caused by nutritional iron deficiency [5]. Widespread iron deficiency in developing countries impacts greatly on national productivity, causing losses of up to 2% of gross domestic product [6].

Vitamin A is another important micronutrient. Reduced immunity, increased morbidity, and risk of death from heightened susceptibility to some infectious diseases, anemia, growth failure, xerophthalmia, and blindness are the major consequences of vitamin A deficiency (VAD) [7,8]. According to the current estimate of the World Health Organization (WHO), the populations of 122 countries have biochemical vitamin A deficiencies of public health significance [7]. Vitamin A deficiency and iron deficiency anemia (IDA) may coexist, but vitamin A can also modulate erythropoiesis, immunity to infectious diseases, and iron metabolism [8]. Thus, VAD, through derangement of iron metabolism, may be considered a leading cause of anemia [8,9]. Vitamins A and E are micronutrient antioxidants. The free-radical-scavenging properties of vitamin E, including inhibition of oxidation of low-density lipoprotein cholesterol in plasma, may protect against chronic diseases [10]. In large observational studies in women, vitamin E intake was associated with lower coronary artery disease rates [11,12].

It has been noted that females are more likely to have micronutrient deficiencies and are more susceptible to the harmful pathophysiologic and socioeconomic consequences of these deficiencies [1]. Worldwide, owing to a diet low in fruits, vegetables, meat, and animal sources of food, women of childbearing years often have an inadequate intake of calcium, iron, folate, zinc, and vitamins A and D. In Asia, about half of the women of childbearing age have IDA [13]. Studies from some Asian and African countries have revealed a high prevalence of biochemical VAD (19%–38%) among pregnant women [14].

The status of micronutrients in lactating or pregnant women may determine infant or child mortality, maternal mortality, growth, and the neurointellectual development of their children; this status can occur through both the direct and the indirect interactions between micronutrients (eg, vitamin A and iron interactions) [1,14,15].

Although IDA and VAD are common micronutrient deficiencies in many developing countries, in many settings, several micronutrient deficiencies exist simultaneously [14,15]. Hence, micronutrient interactions give rise to complex issues that impact etiology, prevention, and treatment [16].

Before the launch of an intervention program that consisted of giving iron-fortified flour to women in the northern Persian Gulf region (2001) [17], more than half of all women of

reproductive age had iron deficiency [18]. The main aim of this cross-sectional, population-based study was to determine the prevalence of vitamins A and E and iron deficiencies among women 15 to 49 years of age in the northern Persian Gulf region, following the flour fortification program. The interaction of iron homeostasis and vitamin A in hemoglobin (Hb) concentration was also investigated. We hypothesized that serum retinol levels may show correlations with Hb concentrations, independent of iron homeostasis.

2. Methods and materials

2.1. Study area and community sampling

Bushehr is a major province of southern Iran. This coastal province has the longest border (700 km) with the Persian Gulf of all Iranian provinces. For our study, women of childbearing age from 8 cities in Bushehr Province were selected between February 2008 and May 2009. Almost all of the households in the 8 cities fell within the ambit of the local health centers of the Bushehr University of Medical Sciences and Health Services.

Based on an interim evaluation for the flour fortification program in Bushehr Province [18], the prevalence of IDA was 7% ($P = .07$). With a confidence level of 95% and absolute precision of 2 percentage points ($d = 0.02$), the sample size was estimated to be around 625 women. By considering a design effect of 2 owing to the cluster sampling strategy of the study, the estimated sample size was doubled ($n = 1250$ women).

A multistage stratified random cluster sampling technique was used to select women in the area covered by each local health center. The first cluster in each local health center was selected using a random number table. The sampling interval was added sequentially to the random number to complete the selection of the remaining clusters in each center. The total number of women selected in the area of each local health center was proportional to the total number of households in that area. The participants were healthy, not pregnant, and between 15 and 49 years of age.

A hand-delivered letter informed the participants about the study. After a preliminary educational session about anemia and micronutrient deficiencies, the women were invited to participate in the screening program. The women who agreed to take part were required to fast overnight before attending one of the local health service centers.

The study was approved by the medical ethical committee of the Bushehr University of Medical Sciences, and written informed consent was obtained from all subjects.

2.2. Survey procedure and data collection

All participants were asked to arrive at the center between 0730 and 0930 hours, after fasting overnight. Upon arrival, information regarding age, marital status, education, socioeconomic status, smoking, obstetric history, drug history (including oral contraceptive use), vitamin, folic acid, and iron supplement intake, and medical history of conditions that could influence iron status was recorded by trained interviewers, using a questionnaire. Participants were considered to be smokers if they smoked cigarettes or used a

hubble-bubble every day. The accepted poverty lines were the thresholds below which households cannot maintain minimal standards of food, clothing, health care, and shelter.

Having removed heavy outer garments and shoes, the women's heights and weights were measured using a stadiometer, and body mass index (BMI) was calculated. Blood pressure was assessed twice at the right arm after a 15-minute rest in the sitting position, using a standard mercury sphygmomanometer.

Venous blood was collected by trained medical staff, and the blood samples (8 mL) were centrifuged at 3000 rpm for 5 minutes. The sera were aliquoted in marked Eppendorf test tubes and kept in the dark at -35°C , until ready for analysis for serum ferritin, transferrin receptor, retinol, and α -tocopherol. EDTA tubes (2 mL) were used for the Hb assay. The complete blood count and Hb component were determined 3 to 5 hours after blood collection using an automated hematology analyzer (Medonic, CA 620, Stockholm, Sweden).

The procedures were conducted at the Persian Gulf Tropical Medicine Research Center, which is attached to the Bushehr University of Medical Sciences.

2.3. Measurements

Serum ferritin levels were measured using an enzyme immunoassay kit (Padtan Elm, Tehran, Iran). The minimum detectable dose of serum ferritin was 0.5 ng/mL, with an intra-assay coefficient of variation of 4.1% and an interassay coefficient of variation of 11.3%.

The soluble (or serum) transferrin receptor (sTfR) levels were measured by a double-sandwich enzyme immunoassay (BioVendor, Modrice, Czech Republic). The analytical limit of detection of the kit was 10 ng/mL, with an intra-assay coefficient of variation of 3.5% to 4.5% and an interassay coefficient of variation of 3.8% to 4.3%.

Owing to limited resources, 727 of 1242 participants were randomly selected for determination of serum retinol (vitamin A) and α -tocopherol (vitamin E) by high-performance liquid chromatography (HPLC; Immundiagnostik AG, Bensheim, Germany). During the precipitation, higher molecular substances were removed. After centrifugation, the supernatant was used for injection into the HPLC system. Separation via HPLC followed an isocratic method at 30°C , using a "reversed phase" column. Detection was performed by an ultraviolet detector at 2 different wavelengths (vitamin A: 325 nm; vitamin E: 300 nm). Quantification was performed with the delivered standard solution; the concentration was calculated via integration of the peak areas into the internal standard calibration mode. The minimum detectable dose of serum vitamins A and E was 0.01 and 1.0 mg/L, respectively, with an intra-assay coefficient variation of 0.9% to 1.0% and 1.1% to 1.9% and an interassay coefficient variation of 3.7% to 4.4% and 4.5% to 5.1%, respectively.

2.4. Definitions

Iron deficiency anemia is defined as a combination of anemia (Hb <12 g/dL) and iron deficiency (serum ferritin <15 ng/mL) [19]. The definition of VAD is based on WHO's definition (vitamin A

<0.70 $\mu\text{mol/L}$). In this regard, vitamin A less than 1.05 $\mu\text{mol/L}$ was considered to be potentially suboptimal vitamin A status [7]. Two thresholds of concentrations of vitamin E (serum α -tocopherol <11.6 and <20 $\mu\text{mol/L}$) were used to define vitamin E deficiency [20].

The ratio of sTfR to the logarithm (to the base 10) of the ferritin concentration (TfR-ferritin index <1) was considered indicative of the anemia of chronic disease [21].

2.5. Statistical analyses

Normal distribution of the data was controlled using the Kolmogorov-Smirnov test. Probability values less than 5% were considered statistically significant. The significance of the difference in the results between the 2 groups was determined by χ^2 analysis using 2×2 contingency tables. A 2-tailed t test was used to compare the values across groups in the presence of a normal distribution. Significant differences were assessed using the Mann-Whitney U test in the absence of normal distribution. Analysis of variance was performed to test differences in biochemical measurements among age groups. The data for serum ferritin levels were defined as the arithmetic mean of the log-transformed data ± 2 SD, raised to the power of 10.

Partial correlation analysis was performed to assess the association between vitamin A concentrations and biochemical variables, with adjustment for age. Multiple linear regression models were used to assess the association between serum vitamin A levels (independent variable) and Hb concentrations (dependent variable). Model 1 was adjusted for age; model 2 was adjusted for age, BMI, employment status, log-transformed ferritin levels, oral contraceptive use, vitamin A, vitamin E, folic acid, and iron supplements. These covariates were included in models because we found that there were significant or marginal differences between the anemic and the healthy groups, according to these parameters. Model 3 was further adjusted for anemia of chronic disease (TfR-ferritin index <1), in addition to all covariates. Because the anemia of chronic disease is a potential mechanism by which vitamin A influences anemia, model 3 was adjusted for it.

All statistical analyses were performed using the Predictive Analytics Software Statistics GradPack 18 (SPSS, Inc, Chicago, IL).

3. Results

The participation rate in the current study was 94.4%. The mean (\pm SD) age of women in the study was 29.83 (± 8.92) years. Of the 1242 participants, 37.8% were between 15 and 25 years old, 34.1% were between 26 and 36 years old, and 28.2% were between 37 and 49 years old.

A total of 392 (32.1%) and 262 (21.4%) were overweight and obese, respectively. The education level of 256 (20.6%) women was elementary school level, and 41 (3.3%) women were illiterate. A total of 255 (20.5%) participants had low socioeconomic status, and 28 (2.3%) women were in absolute poverty. The median number of pregnancies was 3 for the studied population.

Table 1 – The general characteristics including anthropometric data, blood pressure measurements, socioeconomic status, risk factors for IDA, and micronutrients supplementations in women of reproductive age in the northern Persian Gulf

	Total	Healthy	Anemic	P
Age (y)	29.83 (8.92) ^a	29.82 (8.90)	30.01 (9.16)	.830
BMI (kg/m ²)	25.84 (5.57)	25.94 (5.67)	24.86 (4.33)	.055
Systolic blood pressure (mm Hg)	107.10 (12.20)	107.07 (12.34)	107.31 (10.79)	.846
Diastolic blood pressure (mm Hg)	70.62 (8.82)	70.63 (8.94)	70.55 (7.61)	.930
Total duration of lactation (mo)	35.04 (28.15)	34.74 (28.19)	38.38 (27.68)	.330
Marital status; married (%)	71.5	71.9	67.6	.339
Outdoor jobs (%)	21.2	20.5	28.7	.046
Education (%)				
Low	23.9	24.3	20.4	.662
Diploma	62.8	62.6	65.7	
University	13.3	13.2	13.9	
Low socioeconomic status (%)	20.5	20.7	18.5	.599
Parity >2 (%)	35.8	36.2	32.4	.491
History of abortion (%)	20.1	20.2	19.4	.374
Oral contraceptive use (%)	51.6	52.6	41.7	.034
Positive blood donor history (%)	1.7	1.9	0.0	.248
History of GI surgery (%)	2.7	2.6	3.7	.523
Smoking status (%)				
Never	86.7	86.3	90.0	.165
Current	10.7	11.2	5.6	
Past	2.7	2.6	3.7	
Supplements (%)				
Iron	9.5	8.5	20.0	.001
Vitamin A	0.6	0.4	2.6	.066
Vitamin E	1.3	0.9	5.0	.013
Folic acid	6.3	5.5	14.5	.005

GI, gastrointestinal.

^a Data are means (SD).

Table 1 shows the baseline characteristics of the studied population, stratified by the status of anemia. The prevalence of anemia among participants was 8.7% (n = 108). The anemic group contained more employed women than the healthy group (P < .05). Healthy women used more oral contraceptive pills than the anemic women (P < .05), but the anemic group contained more current users of iron, folic acid, and vitamin E supplements (Table 1). There were no differences for the other factors.

Table 2 shows hematologic parameters, serum α -tocopherol, and retinol levels, classified by age group.

Overall, the prevalence of low ferritin and IDA was 25.4% and 4.6%, respectively. The prevalence of anemia, low ferritin, and IDA did not differ significantly across age groups (Table 3). However, there were significant differences in mean concen-

trations of vitamins A and E among the age groups (P < .0001; Table 2). Vitamin A deficiency (<0.7 $\mu\text{mol/L}$) was found in 9 women (1.2%). A total of 22 women (3.0%) had suboptimal levels of vitamin A (<1.05 $\mu\text{mol/L}$).

About 5.9% (n = 43) of the studied population had a serum vitamin E concentration of less than 11.6 $\mu\text{mol/L}$. By changing the cutoff of low vitamin E to less than 20 $\mu\text{mol/L}$, the prevalence of low vitamin E within the studied population increased to 13.9%. The prevalence of VAD and low vitamin E using different cutoffs did not differ significantly across age groups (Table 3).

Although there was no significant difference in vitamin E levels between the anemic (30.48 \pm 11.68 $\mu\text{mol/L}$) and the healthy (32.0 \pm 11.79 $\mu\text{mol/L}$) groups (P > .05), the anemic group

Table 2 – Descriptive statistics of hematologic parameters for iron status, serum α -tocopherol, and retinol levels as classified by age group in women of reproductive age in the northern Persian Gulf

Variables	Total	Age groups (y)			P
		15-25	26-36	37-49	
Ferritin (ng/mL)	31.33 \pm 2.98 ^a	29.92 \pm 2.86	33.11 \pm 2.95	31.33 \pm 3.14	.388
Hb (g/dL)	13.71 \pm 1.26	13.62 \pm 1.09	13.73 \pm 1.20	13.80 \pm 1.50	.101
MCV (fL)	84.07 \pm 7.87	84.07 \pm 7.45	84.14 \pm 8.21	83.99 \pm 8.0	.965
Transferrin receptor ($\mu\text{g/mL}$)	1.65 \pm 1.60	1.59 \pm 1.60	1.62 \pm 1.61	1.78 \pm 1.58	.005
Retinol ($\mu\text{mol/L}$)	2.38 \pm 0.64	2.21 \pm 0.57	2.50 \pm 0.66	2.47 \pm 0.67	<.0001
α -Tocopherol ($\mu\text{mol/L}$)	31.84 \pm 11.78	29.42 \pm 10.04	32.49 \pm 11.80	34.26 \pm 13.21	<.0001

MCV, mean corpuscular volume.

^a Data are means \pm SD, except for ferritin and transferring receptor, which are presented as geometric means \pm SD.

Table 3 – Prevalence of vitamins A and E and iron deficiencies, anemia, and IDA as classified by age group in women of reproductive age in the northern Persian Gulf

Variables	Total	Age groups (y)			P
		15-25	26-36	37-49	
VAD					
<0.70 $\mu\text{mol/L}$	1.2 (9) ^a	1.8 (5)	1.3 (3)	0.5 (1)	.208
<1.05 $\mu\text{mol/L}$	3.0 (22)	4.6 (13)	1.8 (4)	2.3 (5)	.134
Vitamin E deficiency					
<11.6 $\mu\text{mol/L}$	5.9 (43)	6.0 (17)	5.8 (13)	5.9 (13)	.995
<20 $\mu\text{mol/L}$	13.9 (101)	14.5 (41)	13.5 (30)	13.6 (30)	.926
Iron deficiency: ferritin <15 ng/mL	25.4 (315)	24.4 (114)	25.3 (107)	26.9 (94)	.728
Anemia: Hb <12 g/dL	8.7 (108)	9.0 (42)	8.3 (35)	8.9 (31)	.934
IDA: ferritin <15 ng/mL and Hb <12 g/dL	4.6 (57)	4.3 (20)	4.0 (17)	5.7 (20)	.487

^a Data are percentage (numbers).

had lower vitamin A levels than did the healthy group ($2.13 \pm 0.66 \mu\text{mol/L}$ versus $2.41 \pm 0.64 \mu\text{mol/L}$; $P < .0001$).

Table 4 shows correlations between serum vitamin A levels and Hb concentration in different models. An age-adjusted concentration of vitamin A was significantly correlated with Hb concentration ($\beta = .18$, $P < .0001$, model 1).

In multiple regression analysis, after adjustment for age, BMI, employment status, log-transformed ferritin concentration, vitamin E, folic acid, iron supplement, or oral contraceptive use, the serum vitamin A level was significantly correlated with Hb concentration (model 2; Table 4). Parameter estimates of this correlation remained after adjusting for all confounders, in addition to the anemia of chronic disease (model 3). These associations remained unchanged after excluding subjects with IDA (models 1-3; Table 4).

4. Discussion

According to the accepted cutoffs [7], the prevalence of VAD (<0.70 $\mu\text{mol/L}$) and suboptimal vitamin A status (<1.05 $\mu\text{mol/L}$) among women of childbearing age in the northern Persian Gulf region was found to be 1.2% and 3.0%, respectively. Vitamin A status is considered a public health problem if more than 2% of the population has serum retinol levels of 0.70

$\mu\text{mol/L}$ or lower [14]. Thus, based on the results of the current study, VAD may not be considered a public health problem among women of reproductive age in the northern Persian Gulf region.

The mean serum retinol concentration of our study population was higher than the values reported for Kuwait [22], Korea [23], France [24], and Saudi Arabia [25], but it was comparable with those found in Japan [26], Malaysia [27], and the United States [28]. Bagchi et al [29] found that 7 of the 10 studied populations from different countries had mean or median values in the upper part of the reference range for retinol. Tee and Khor [27] recommended that the suggested 20 to 49 $\mu\text{g/dL}$ (0.70–1.71 $\mu\text{mol/L}$), considered an “acceptable” serum vitamin A level by the US Interdepartmental Committee on Nutrition for National Defense, should be reconsidered. Updated reference ranges for 98% to 99% of the US population, based on National Health and Nutrition Examination Survey III, were determined [28]. Accordingly, the Centers for Disease Control and Prevention in Atlanta recommended a reference range of 25 to 103 $\mu\text{g/dL}$ (0.87–3.59 $\mu\text{mol/L}$) for vitamin A in subjects aged 20 to 49 years [28].

There are relatively few nationally representative studies on vitamin E status and the distribution of serum concentrations of α -tocopherol [20]. In medical literature, different thresholds are used to estimate vitamin E deficiency [20]. In

Table 4 – Multiple linear regression analysis for the association between serum vitamin A levels (independent variable) and Hb concentration (dependent variable) in different models

	Hb			
	All ^a		Excluded IDA ^b	
	β	P	β	P
Model 1 (adjusted for age)	.18	<.0001	.15	<.0001
Model 2 (adjusted for age, covariate ^c , and log-transformed ferritin)	.09	.024	.10	.034
Model 3 (adjusted for age, covariate ^c , log-transformed ferritin, and the anemia of chronic disease ^d)	.09	.026	.09	.038

^a All participants of the studied population were included.

^b Those with iron deficiency anemia were excluded.

^c The covariates included BMI, employment status, oral contraceptive use, vitamin A, vitamin E, folic acid, and iron supplements.

^d The anemia of chronic disease was defined as (TfR-ferritin index <1).

the National Health and Nutrition Examination Survey (1999–2000), the prevalence of vitamin E deficiency was low (21.0%, using a cutoff of $<20 \mu\text{mol/L}$) among women 20 years or older in the United States, despite the fact that the diets of most Americans provide less than the recommended dietary allowance levels of vitamin E [20]. In the current study, we found that 13.9% of nonpregnant women of reproductive age had low vitamin E levels ($<20 \mu\text{mol/L}$). Using the most common cutoff for vitamin E ($<11.6 \mu\text{mol/L}$), the prevalence of low vitamin E changed to 5.9%. Thus, most nonpregnant women of reproductive age in the northern Persian Gulf region have adequate vitamin E levels.

The worldwide prevalence of anemia in nonpregnant women (1993–2005) was 30.2% [5], whereas in the eastern Mediterranean region, the prevalence was 32.4% [29]. In our previous study of the northern Persian Gulf (1999), the prevalence of anemia, iron deficiency, and IDA among women of reproductive age was 18.1%, 53.3%, and 12.8%, respectively [18]. By using the same cutoff for serum ferritin as we used in our previous study (serum ferritin <10), the current prevalence of anemia, iron deficiency, and IDA is 8.7%, 17.7%, and 4%, respectively. Comparing the current data with those reported in our previous study reveals major drops in the prevalence of anemia, iron deficiency, and IDA among women of reproductive age. These figures show that the status of anemia in nonpregnant women of reproductive age in the northern Persian Gulf changed from a moderate to a mild public health problem, according to the WHO classification.

The surprising decrease in iron deficiency in the northern Persian Gulf region arises from an intervention program consisting of iron-fortified flour, which was launched on May 31, 2001, by the Iranian Ministry of Health and Medical Education [17]. The iron-fortified flour premix included 30 ppm ferrous sulfate and 1.5 ppm folic acid. This program may serve as a practical model for other developing countries considering flour fortification as part of their public health strategy to combat micronutrient deficiencies [30].

We found a significant correlation between retinol levels and Hb concentrations. A high correlation between serum or plasma retinol and Hb has been reported from many different countries [8]. A high prevalence of anemia among populations with VAD has been reported in population-based studies from developing countries [8]. In the current study, serum retinol concentrations were also positively correlated with serum ferritin concentrations. The association of VAD and IDA has been documented in many clinical and population-based studies [31]. Experimental animal studies show that vitamin A has an important role in hematopoiesis [32,33]. Although diverse mechanisms have been described for vitamin A and iron interactions, the molecular mechanisms of these interactions are not clear. It was found that vitamin A supplementation in children with poor vitamin A and iron status mobilized iron from hepatic iron stores by increasing circulating erythropoietin [34]. Iron regulatory proteins, as central regulators of iron homeostasis, have been suggested as a mechanistic link between the regulation of iron homeostasis and vitamin A [35].

In the current study, anemic women of reproductive age had significantly lower retinol concentrations than do healthy

women. Even excluding those with IDA, we found that serum vitamin A levels were positively associated with Hb concentrations after adjustment for ferritin levels in multiple regression analysis. This finding confirms our hypothesis that low vitamin A levels may contribute to anemia, independent of iron status. However, it should not be ignored that, mechanistically, low vitamin A affects iron use by impairing the use of iron stores [31]. In experimental animal studies, VAD was associated with increased iron accumulation in the liver and spleen [32,33].

Modulation of erythropoiesis, modulation of immunity to infectious diseases, and, hence, the anemia of infection as a form of the anemia of chronic disease are potential mechanisms by which vitamin A influences anemia [8]. In epidemiologic surveys, calculation of the ratio of sTfR to serum ferritin (sTfR/log ferritin or sTfR-ferritin index) is useful for estimating total body storage of iron, especially in the presence of coexisting chronic disease [34]. An sTfR-ferritin index less than 1 is suggestive of the anemia of chronic disease, but patients may be considered to have combined IDA and the anemia of chronic disease when they have an sTfR-ferritin index greater than 2 [4]. In our study, the association of vitamin A levels with Hb concentrations persisted when further adjustment was done for the anemia of chronic disease by entering sTfR-ferritin index in multiple regression models. Low vitamin A may thus be considered a cause of anemia [8]. Further studies are needed to elucidate the biological and pathophysiologic mechanisms that control vitamin A–anemia interactions, including the effect of vitamin A on the pathophysiology of iron stores.

The current study is the largest population-based study for iron status in the northern Persian Gulf region. A major limitation of our study was the absence of dietary assessment of participants. Although we used sTfR-ferritin index as an indicator of the anemia of chronic disease, we did not assess C-reactive protein to evaluate the role of chronic inflammation in our results. Owing to resource constraints, we did not measure vitamins A and E in all participants. However, there were no significant differences between these groups in respect of their baseline characteristics and data relevant to vitamins A and E. Serum cholesterol also was not measured to determine α -tocopherol/cholesterol ratio. The measurement of lipid-standardized plasma vitamin E may be important because concentrations of vitamin E are dependent on concentrations of lipids [20,22].

In conclusion, VAD ($<0.70 \mu\text{mol/L}$) and vitamin E deficiency ($<11.6 \mu\text{mol/L}$) may not be considered a public health problem among women of reproductive age in the northern Persian Gulf region. Because serum vitamin A levels were positively associated with Hb concentrations after adjustment for ferritin levels and the anemia of chronic disease in multiple regression analysis, low vitamin A levels may contribute to anemia, independent of iron status. As the prevalence of iron deficiency begins to show a downward trend, other causes of anemia may become proportionately more prominent [36]. Therefore, a concurrent integrated approach should be planned to combat other micronutrient deficiencies and contributing complex factors in anemia in the northern Persian Gulf region.

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